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Proof of the Mysterious Efficacy of Ginseng: Basic and Clinical Trials: Metabolic Activation of Ginsenoside: Deglycosylation by Intestinal Bacteria and Esterification with Fatty Acid

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Abstract. Orally ingested ginsenoside passes through the stomach and small intestine without decomposition by either gastric juice or liver enzymes into the large intestine, where ginsenoside is deglycosylated by colonic bacteria followed by transit to the circulation. Colonic bacteria cleave the oligosaccharide connected to the aglycone stepwise from the terminal sugar to afford the major metabolites, 20*S*-protopanaxadiol 20-*O*-β-D-glucopyranoside (M1) and 20*S*-protopanaxatriol (M4). These metabolites are further esterified with fatty acids. The resultant fatty-acid conjugates are still active molecules that are sustained longer in the body than parental metabolites. Accumulating evidence strongly suggests that ginsenoside is a prodrug that is activated in the body by intestinal bacterial deglycosylation and fatty acid esterification.

Keywords: ginsenoside, metabolic activation, intestinal bacteria, deglycosylation, fatty acid esterification

Introduction

In the latter of the 20th century, the pharmaceutical studies on ginseng have started in parallel with structural elucidation of its ingredients, in order to give scientific grounds to the medical efficacy of ginseng. So far, numerous researchers have contributed to the accumulation of evidence that ginsenoside is responsible for the pharmacological effects of ginseng. Indeed, ginsenoside itself exerted various pharmacological activities, by directly being added to cell cultures in vitro or by being intraperitoneally or intravenously injected to experimental animals. These results led to the misunderstanding that intact ginsenoside might be the real active principle in the body. However, Kobashi et al. have proposed the concept that plant glycoside acts as a prodrug that is metabolized to the active form by intestinal bacterial deglycosylation (1, 2). We have revealed that the anticancer activities of ginsenoside after oral administration are based on its metabolites formed by intestinal bacterial deglycosylation (3, 4) and fatty-acid esterification (5 – 7). These data have contributed evidence to their hypothesis and also expanded the research field of plant glycosides (8). This review describes the metabolic activation of ginsenoside in the body.

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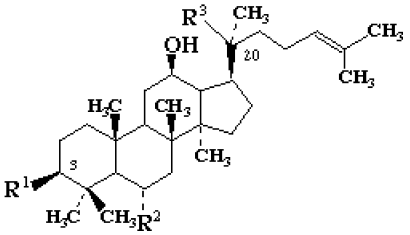
Deglycosylation of ginsenoside by intestinal bacteria

Ginseng is orally ingested, in general. Therefore, its ingredients must meet gastric juice, digestive and bacterial enzymes in the intestines. Ginsenoside is hardly decomposed by gastric juice with the exception of slight oxygenation (9); however, the oral bioavailabilities of intact ginsenosides from the intestines are extremely low (Rb_1, 0.1% to 4.4%; Rb_2, 3.7%; Rg_1, 1.9% to 18.4%) (10 – 12). In spite of poor absorption of ginsenoside, radioisotope assay revealed that serum radioactivity in rats orally given Rb_2 together with its radioactive derivative was 3 times higher than the serum Rb_2-level determined by HPLC (11).

Our results from incubation of Rb_1 with the contents of mouse gastrointestinal tract showed that ginsenoside is metabolized to 20*S*-protopanaxadiol 20-*O*-β-D-glucopyranoside (M1, Table 1) only by the contents of the large intestine (13). This phenomenon was also observed in the mice orally administered with Rb_1. With

Table 1. Chemical structures of ginsenosides and their metabolites formed by intestinal bacteria

Ginsenoside	R^1	R^2	R^3
			
Major ginsenoside			
20*S*-Protopanaxadiol type:			
Rb_1	O-Glc^2-^{1}Glc	H	O-Glc^6-^{1}Glc
Rb_2	O-Glc^2-^{1}Glc	H	O-Glc^6-^{1}Arap
Rc	O-Glc^2-^{1}Glc	H	O-Glc^6-^{1}Araf
Rd	O-Glc^2-^{1}Glc	H	O-Glc
20*S*-Protopanaxatriol type:			
Re	OH	O-Glc^2-^{1}Rha	O-Glc
Rg_1	OH	O-Glc	O-Glc
Intestinal bacterial metabolite			
20*S*-Protopanaxadiol type:			
M10 (Rd)	O-Glc^2-^{1}Glc	H	O-Glc
M9 (Gp-XVII)	O-Glc	H	O-Glc^6-^{1}Glc
M7 (Mb)	O-Glc	H	O-Glc^6-^{1}Araf
M6	O-Glc	H	O-Glc^6-^{1}Arap
M13 (Gp-LXXV)	OH	H	O-Glc^6-^{1}Glc
M5 (F_2)	O-Glc	H	O-Glc
M3 (Mc)	OH	H	O-Glc^6-^{1}Araf
M2 (C-Y)	OH	H	O-Glc^6-^{1}Arap
M1 (C-K)	OH	H	O-Glc
M12 (aglycone)	OH	H	OH
20*S*-Protopanaxatriol type:			
M8 (Rh_1)	OH	O-Glc	OH
M11 (F_1)	OH	OH	O-Glc
M4 (aglycone)	OH	OH	OH

Glc, β-D-glucopyranose; Arap, α-L-arabinopyranose; Araf, α-L-arabinofuranose; Rha, α-L-rhamnopyranose; Gp, gypenoside; C-K, compound K; C-Y, compound Y.

the passage of time after administration, Rb_1 was detected in the stomach and small intestine followed by M1 in the caecum and colorectum (13). These results indicate that intestinal bacteria metabolize Rb_1 to M1. Akao et al. detected M1, which they called compound K, in the plasma of gnotobiotic rats orally administered with Rb_1 (14). Recently, Tawab et al. provided evidence that M1, M8, and M11 may reach the circulation in humans after ginseng ingestion (15).

Intestinal bacteria cleave the oligosaccharides connected to the C-3 or C-20 hydroxyl group of the aglycone stepwise from the terminal sugar (16, 17). The main metabolic pathways are supposed to be as follows: protopanaxadiol-type, $Rb_1 \rightarrow$[M10 (Rd)$\rightarrow$M5 (F_2) or M9$\rightarrow$M13]$\rightarrow$M1, $Rb_2 \rightarrow$M6$\rightarrow$M2$\rightarrow$M1, and Rc$\rightarrow$M7$\rightarrow$M3$\rightarrow$M1 (M1 is gradually hydrolyzed to M12); protopanaxatriol-type, Re$\rightarrow Rg_1 \rightarrow$M11 ($F_1$) or M8 ($Rh_1$)$\rightarrow$M4 (Table 1). Many kinds of bacteria including *Prevotella oris* (18), *Eubacterium* A-44 (14), *Bifidobacterium* K506 (17), *Bacteroides* JY6 (17), and *Fusobacterium* K-60 (17) seem to cooperatively metabolize ginsenoside.

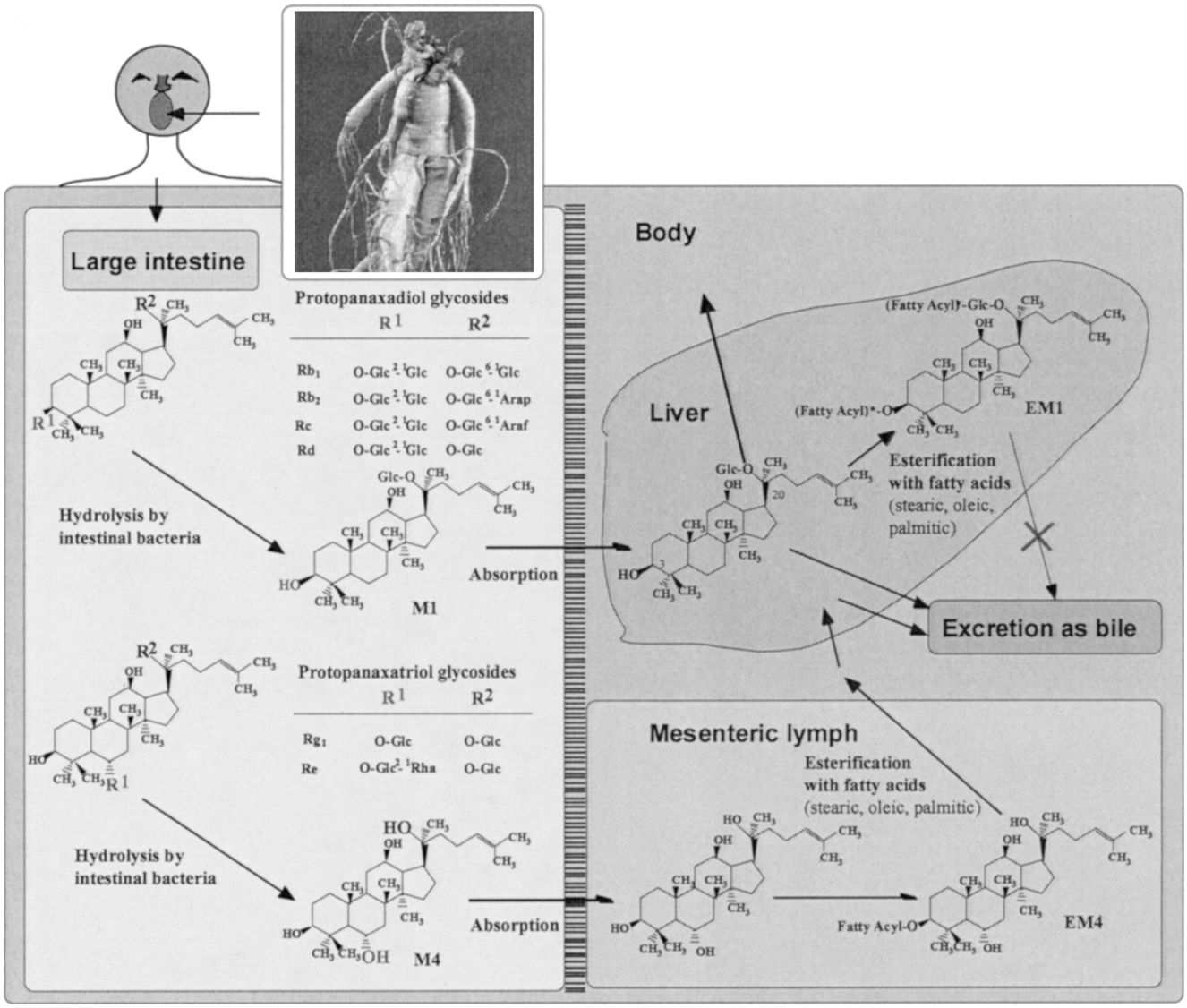


Fig. 1. Putative metabolic pathways of ginsenosides in the body after oral administration. Ginsenosides are deglycosylated to M1 or M4 by intestinal bacteria followed by absorption into the blood or the mesenteric lymphatics. Although M1 is mostly excreted as bile, some M1 may be esterified with fatty acids at C-3 of the aglycone moiety or C'-6 of the glucose moiety in the liver. EM1 is not excreted in the small intestine and so it is accumulated in the liver longer than M1. On the contrary, most M4 is esterified with fatty acids and accumulated in the tissues including the liver and lung followed by excretion of esterified M4 (EM4) as bile.

Esterification of intestinal bacterial metabolite with fatty acid

Following intravenous administration of M1 to rats and mice, it rapidly disappeared from the circulation ($t_{1/2\alpha}$, 3 min; $t_{1/2\beta}$, 23 min; AUC, 2815 min$\cdot\mu$g/ml) and instead, increased in the liver. The hepatic M1-level peaked at 10 min (C_{max}, 65% recovery) and thereafter gradually disappeared with time ($t_{1/2\alpha}$, 25 min; $t_{1/2\beta}$, 75 min; AUC, 481.4 h$\cdot\mu$g/g). Although most M1 was excreted as bile, approximately 24 mol% of dosed M1 was esterified with fatty acids in the liver without structural variation. Esterified M1 (EM1) was not excreted in the small intestine and so EM1 was accumulated in the liver longer than M1, thus suggesting that metabolic regulation differs between M1 and EM1. Mass spectrometry analysis defined EM1 as a mixture of various fatty acid M1 mono-esters, including stearate, oleate, or palmitate (5). The fragment pattern of EM1 suggests that fatty acid is linked to M1 at C-3 of the aglycone moiety or C'-6 of the glucose moiety.

In the case of M4 (7), orally ingested M4 was

completely absorbed from the small intestine into the mesenteric lymphatics. Then rapid fatty acid esterification of M4 occurred at C-3 of the aglycone moiety. Esterified M4 (EM4) spread to other organs in the body followed by its excretion as bile. Inconsistent with M1, the selective accumulation of M4 in the liver after its intravenous administration was not observed. The structural difference between M1 and M4 is the glucose moiety connected at C-20 of the aglycone (Table 1). Hepatocytes are shown to recognize glucose moiety via a receptor (19, 20). This specific function of hepatocytes must be partly associated with the selective accumulation of M1 into the liver. Based on these results, putative metabolic pathways of ginsenoside after oral administration are proposed in Fig. 1.

To our knowledge, there have been few reports on animal fatty acid triterpene esters, except for 2 reports by Tabas et al. who isolated the triterpene esters containing stearate and palmitate from the liver of rabbits and humans (21). They hypothesized that the origin of fatty acid triterpene esters may be via dietary absorption of plant triterpenes followed by fatty acid esterification of the triterpene in animal tissues (22). The detection of EM1 and EM4 in the tissues is the first evidence for their hypothesis.

Metabolic regulation of ginsenoside metabolites (M1 and M4) differed from those glycosides which are conjugated with glucronic acid (ex., glycyrrhetic acid and baicalein, the sapogenins of glycyrrhizin and baicalin) (1).

Pharmacological activity of ginsenoside metabolite

An issue raised here is whether ginsenoside metabolites are active or not. The results from the in vivo and in vitro comparative examinations using ginsenoside and its intestinal bacterial metabolite are as follows (2, 3): When administered orally (ginsenoside is hydrolyzed to metabolite by intestinal bacteria), both ginsenoside and metabolite were effective in inhibiting tumor metastasis. However, the activity of orally ingested ginsenoside was correlated with the ginsenoside-hydrolyzing potential of intestinal bacteria (23). In the case of intravenous administration (metabolite is not produced

Table 2. Pharmacological activity of ginsenoside metabolites cited in literature

Ginsenoside metabolite	assay condition	pharmacological potential (Reference No.)
Intestinal bacterial metabolite		
20(*S*)-Protopanaxadiol type:		
M3 (Mc)	in vitro	anti-inflammatory activity (17)
M1 (C-K)	in vitro	inhibitor of tumor cell proliferation (2, 27)
		inhibitor of tumor-induced neovascularization (25)
		inducer of apoptotic cell death (26)
		regulator of tumor cell cycle (27)
		anti-inflammatory activity (17)
	in vivo	inhibitor of tumor growth (25)
		inhibitor of tumor metastasis (2, 23)
		inhibitor of carcinogenesis (13)
		regulator of hypothalamo-pituitary-adrenal axis (28)
M12 (aglycone)	in vitro	inhibitor of *Helicobacter pylori* (29)
		inhibitor of tumor growth (17)
20(*S*)-Protopanaxatriol type:		
M4 (aglycone)	in vitro	inhibitor of catecholamine secretion (30)
		inhibitor of tumor cell proliferation (3)
	in vivo	inhibitor of tumor metastasis (3)
Fatty acid ester of intestinal bacterial metabolite		
20(*S*)-Protopanaxadiol type:		
Fatty acid M1 ester (EM1)	in vitro	immunomodulator (6)
	in vivo	inhibitor of tumor growth (5, 6)
		inhibitor of tumor metastasis (5, 6)
20(*S*)-Protopanaxatriol type:		
Fatty acid M4 ester (EM4)	in vitro	potentiator of natural killer activity (7)

from ginsenoside), not ginsenoside but metabolite was effective (2, 3). Tumor invasion into extracellular matrices and basement membranes is a crucial step in the complex multistage process that leads to the metastatic formation. Under in vitro conditions, slight or no inhibition of tumor invasion was seen using ginsenoside, whereas the metabolite even at non-cytotoxic concentrations inhibited tumor invasion more effectively than ginsenoside (2, 3). These findings suggest that the action of bacterial metabolites primarily mediates the antimetastatic effects by orally ingested ginsenoside. This concept agrees with the pharmacokinetic findings: not ginsenosides but their bacterial metabolites are absorbed from the intestines after oral administration of ginsenosides.

The in vitro cytotoxicity of metabolites was attenuated by fatty acid esterification (6), implying that the esterification is a detoxification reaction, just as cholesterol esterification is shown to prevent the cytotoxicity of excess intracellular cholesterol (24). Metabolites did not directly affect tumor growth in vitro (6), whereas they stimulated lymphocytes to become cytotoxic to tumor cells (6, 7). Concerning the pharmacokinetics, both M1 and EM1 were selectively taken up into the liver soon after intravenous administration. Thereafter, M1 was cleared immediately from the liver; however, EM1 was retained in the liver at a level of more than 25% of the administered dose for 24 h after administration (6). These findings suggest that fatty acid esterification of intestinal bacterial metabolite of ginsenoside potentiates the antitumor activity of the parental metabolites through delay of the clearance and through immunostimulation.

The pharmacological activities of ginsenoside metabolites are summarized in Table 2.

Conclusion

Orally ingested ginsenoside is activated by intestinal bacterial deglycosylation followed by fatty acid esterification. Therefore, the deglycosylation process of ginsenoside is crucial for its pharmacological expression. Intestinal bacteria, however, are very changeable in dependence of host conditions, including diet, health, and even stress. Indeed, bacterial ginsenoside-hydrolyzing potentials have been shown to differ among humans (18) and experimental mice (13). Therefore, it is easily hypothesized that the individual differences in bacterial ginsenoside-hydrolyzing potentials may affect ginseng efficacy. Fermented ginseng products containing ginsenoside metabolites may have merit for standardizing ginseng efficacy.

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